Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (8)

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Sport und Buch Strauß, Köln, 2000

I.BAILLOUX, S.DE BARROS, A.LAHAUSSOIS, J.-C.MATHURIN, J.DE CEAURRIZ:
LC-MS and LC-MS/MS in Doping Control Analysis of Synthetic Glucocorticoids
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (8). Sport und Buch Strauß, Köln, (2000) 203-207

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LC-MS and LC-MS/MS in doping control analysis of synthetic glucocorticoids

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INTRODUCTION

The use of synthetic glucocorticoids as therapeutic or doping agents in sport is underestimated probably because it is only subjected to restriction from a regulatory point of view. Moreover, many of these compounds are thermally labile and their volatility is too low for direct GC analysis. Consequently their detection in routine analysis for anti-doping control have been often neglected. HPLC offers the advantage that the corticosteroids can be analysed without thermal degradation and derivatisation.

For control purposes, the LNDD developed a LC-MS screening procedure for these substances involving an on-line SPE pre-purification of urine samples. For confirmatory purposes, extracts from urine samples were obtained according to an off-line procedure involving SPE and further analysed using an ion trap LC-MS/MS instrument. This procedure has been applied to the cycling race « Tour de France 1999 » and examples of analysis were presented.

EXPERIMENTAL

HPLC and MS conditions

Screening

Instruments : HPLC 1100 (Hewlett Packard), colonne Eclipse XDB C8 (150 mm x 2.1 mm - 5µm).

on-line SPE Prospekt (Eurosep), colonne Hysphère C8.

Mobile Phase : solvent A: $HCOONH_4$ 10mM/l

solvent B: CH_3CN (30% 0-4 min, 40% 10 min, 70% 20 min, 30% 25 min)

Flow rate : 0.25 ml/min. Column temperature : 25°C

Ionisation: ESI in positive mode (MSD 1100, Hewlett Packard)

Nebuliser pressure : 30 psig, Gas temperature : 300°C, Flow gas : 10 L/min

Capillary voltage : 6000V, Acquisition mode : SIM

Confirmation

LC and MS conditions were established depending on each molecule (see table 1)

Instrument : LC1100 (Hewlett Packard)/Esquire (Brücker)

Sample preparation

Screening sample

2 ml of urine (9<pH<10)
+ ISTD (methyltestosterone)

centrifugation

SPE on-line LC/MS

Column C8
Wash water
Elute with mobile phase

Confirmation sample

4 ml of urine (9<pH<10)
+ ISTD (methyltestosterone)

centrifugation

SPE

Column C18
Elute CH_2Cl_2

LC/MS/MS 20 µl

RESULTS

Screening

The parameters of analysis of 15 synthetic corticosteroids and two natural corticosteroids (hydrocortisone and cortisone) are described in table 1. The LOD was estimated at 2 ng/ml or less for each compound except for triamcinolone (about 10 ng/ml).

The results of the screening for two samples obtained during the « Tour de France 1999 » are shown in figures 1 and 2. Sample A contained dexamethasone (estimated concentration : 100 ng/ml) and sample B contained triamcinolone acetonide (estimated concentration : 0.4 ng/ml).

Confirmation

LC-MS/MS parameters of 15 synthetic corticosteroids are reported in table 2. The ionisation mode, the mobile phase and the amplitude of fragmentation for MS/MS sequence have been optimised.

Mass spectra of a blank urine spiked with a reference of triamcinolone acteonide (1 ng/ml) and the urine sample B are shown in figures 3.

Three diagnostic ions were used for the identification of the molecule : m/z 415, m/z 397, m/z 339. Variations in the relative abundance of ions m/z 397 and m/z 339 between the reference and the sample were less than 15 % for these ions.

CONCLUSION

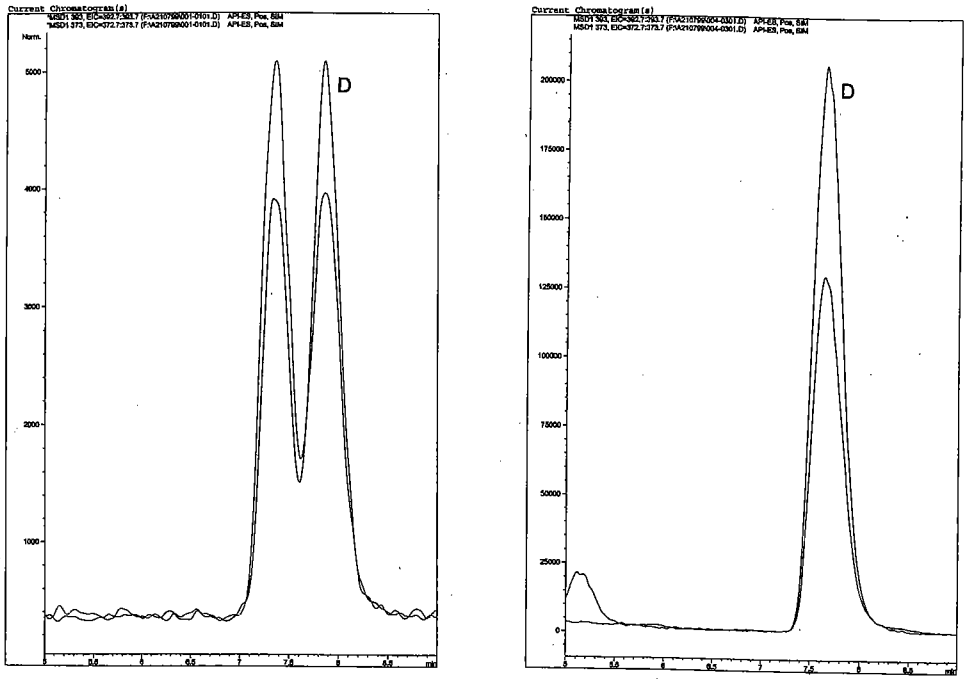
LC-MS combined with on-line SPE can be regarded as a practical technique for the screening of synthetic glucocorticoids in urine. The analysis time is reduced by the use of on-line SPE (about 45 min per sample). However, the use of shorter HPLC columns would permit this time to be reduce to about 25 minutes. Moreover, LC-MS/MS analysis is a must for confirmation of corticosteroids at low concentrations. Out of the 93 urine samples from the "Tour de France" which have been analysed, triamcinolone acetonide, betamethasone and dexamethasone account for 31%, 3.2% and 1% of glucocorticoids, respectively. It is thought that further attention should be paid to the routine analysis of synthetic corticoids despite the positivity of samples remains dependent on therapeutic notification.

Molecules	Specific ions	Relative Retention Time (min)	CID Voltage (V)
Triamcinolone	395.2 - 375.2	0.168	70
Prednisolone	361.2 - 343.2	0.248	70
Cortisol	363.2	0.260	70
Fludrocortisone	381.2	0.282	70
Cortisone	361.2	0.290	70
Methylprednisolone	375.2 - 357.2	0.392	70
Betamethasone	393.2 - 373,2	0.437	70
Dexamethasone	393.2 - 373.2	0.461	70
Beclomethasone	409.2 - 391.2	0.552	70
Desonide	417.2 - 399.2	0.602	70
Triamcinolone acetonide.	435.2 - 415.3	0.628	70
Flunisolide	435.2 - 417.3	0.657	70
Fluocortolone	377.2 - 359.2	0.740	70
Fluorometholone	377.2 - 357.2	0.767	70
Budesonide	431.2 - 413.2	0.990-0.996	73
Medrysone	345.2 - 327.2	1.128	100
Cortivazol	531.3 - 443.3^{+}	1.529	240
Methyltestosterone	303.2	1 (16.7 min)	73

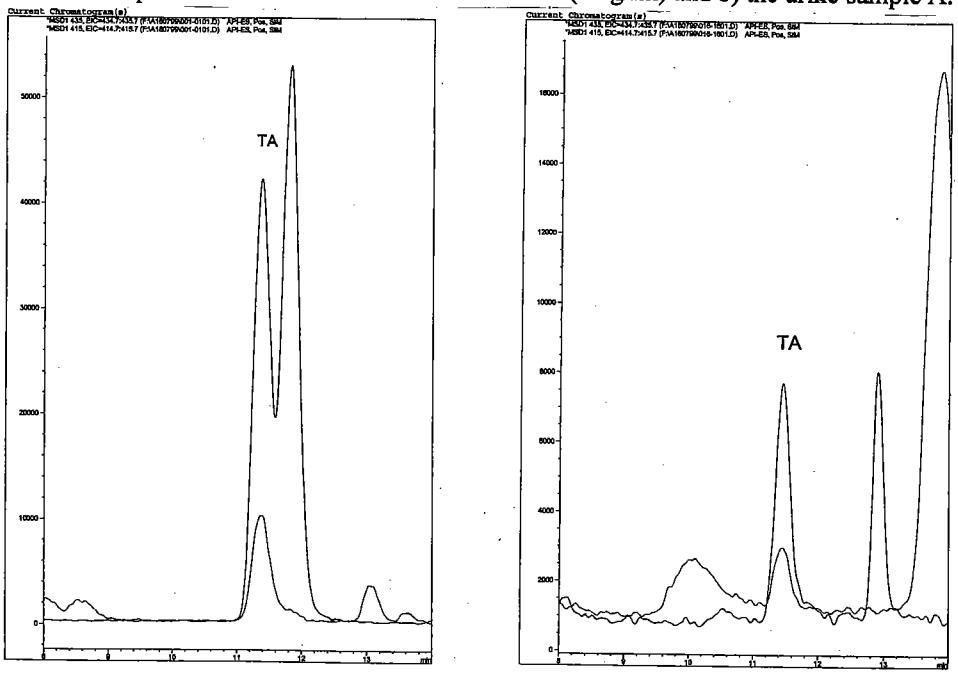
Table 1 : Experimental conditions for LC/MS screening in SIM mode

Molecules	Source/Mode	Mobile phase (60/40)	Ampl. frag.(V)
Triamcinolone	ESI +	Ammon. formate 10mM/l /CH_3CN	0.6
Prednisolone	APCI +	Acetic acid 0.01% / CH_3CN	1.3
Fludrocortisone	APCI +	Acetic acid 0.1% / CH_3CN	1.55
Methylprednisolone	ESI +	Ammon. formate 10mM/l / CH_3CN	0.55
Betamethasone	APCI +	Acetic acid 0.01% / CH_3CN	0.95
Dexamethasone	APCI +	Acetic acid 0.01% / CH_3CN	0.93
Beclomethasone	APCI +	Water / CH_3CN	0.95
Desonide	ESI +	Ammon. formate 100mM/l / CH_3CN	0.57
Triamcinolone acetonide	APCI +	Ammon. formate 50mM/l / CH_3CN	1.05
Flunisolide	ESI +	Ammon. formate 10mM/l / CH_3CN	0.65
Fluocortolone	APCI -	Acetic acid 0.01% / CH_3CN	1.60
Fluorometholone	APCI +	Acetic acid 0.01% / CH_3CN	1.00
Budesonide	APCI +	Acetic acid 0.01% / CH_3CN	1.12
Medrysone	ESI +	Ammon. formate 10mM/l / CH_3CN	1.25
Cortivazol	ESI +	Ammon. formate 50mM/l / CH_3CN (30/70)	2.2

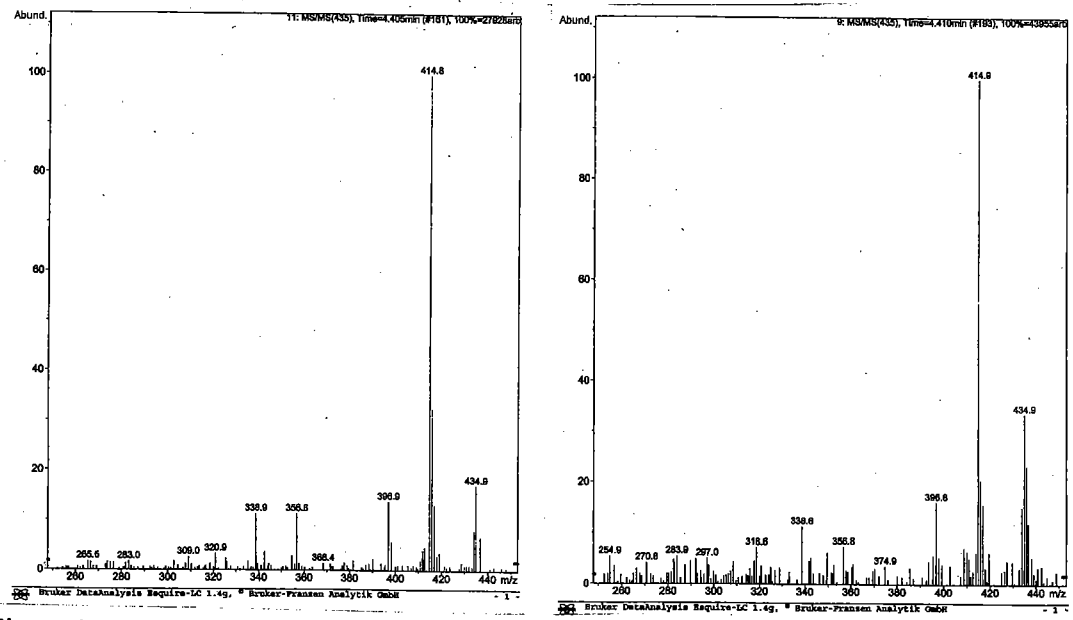
Table 2 : Experimental conditions for LC/MS/MS confirmation



Figures 1 :LC-MS chromatogram (extracted ions m/z 393 and m/z 373) of
a) a calibration point with a reference of dexamethasone (2 ng/ml) and b) the urine sample A.



Figures 2 : LC-MS chromatogram (extracted ions m/z 435 and m/z 415) of
a) a calibration point with a reference of triamcinolone acetonide (2 ng/ml) and b) the urine sample B.



Figures 3 : MS/MS mass spectra of a) triamcinolone acteonide in urine (1 ng/ml) and b) the urine sample B.